

PATENT/Docket No. PC11050A  
Appl. No. 09/989,933  
Filing Date: November 21, 2001

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AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-29 (Canceled)

30. (Currently amended) An attenuated bovine viral diarrhea virus wherein said virus carries in the viral genome:

- (a) a mutated N<sup>pro</sup> coding sequence comprising an intact 5' region of at least about 36 base pairs, wherein said mutated N<sup>pro</sup> coding sequence encodes an inactive N<sup>pro</sup> protein; and
- (b) a sequence coding for a monomeric bovine ubiquitin wherein the ~~ubiquitin~~ ubiquitin coding sequence is operably placed between the 3' end of said mutated N<sup>pro</sup> coding sequence and the 5' end of the coding sequence for the viral core protein.

31. (Currently amended) ~~The~~ An attenuated bovine viral diarrhea virus ~~of claim 30~~, comprising a genomic nucleic acid sequence as set forth in SEQ ID NO: 11, or a degenerate variant thereof wherein said degenerate variant encodes the same amino acid sequence encoded by SEQ ID NO: 11, wherein said virus carries in the viral genome:

- (a) a mutated N<sup>pro</sup> coding sequence comprising an intact 5' region of at least 36 base pairs, wherein said mutated N<sup>pro</sup> coding sequence encodes an inactive N<sup>pro</sup> protein; and
- (b) a sequence coding for a monomeric bovine ubiquitin wherein the ubiquitin coding sequence is operably placed between the 3' end of said mutated N<sup>pro</sup> coding sequence and the 5' end of the coding sequence for the viral core protein.

32. (Currently amended) The attenuated bovine viral diarrhea virus of claim 30 wherein the mutated N<sup>pro</sup> coding sequence comprises an intact 5' region of at least ~~about 310 base pairs~~ pairs.

33. (Currently amended) An isolated nucleic acid molecule comprising the genomic sequence of an attenuated bovine diarrhea virus, wherein said virus carries in the viral genome:

- (a) a mutated N<sup>pro</sup> coding sequence comprising an intact 5' region of at least ~~about 36 base pairs~~, wherein said mutated N<sup>pro</sup> coding sequence encodes an inactive N<sup>pro</sup> protein and

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(b) a sequence coding for a monomeric bovine ubiquitin wherein the ubiquitin coding sequence is operably placed between the 3' end of said mutated N<sup>pro</sup> coding sequence and the 5' end of the coding sequence for the viral core protein.

34. (Currently amended) The isolated nucleic acid molecule of claim 33 wherein the mutated N<sup>pro</sup> coding sequence comprises an intact 5' region of at least ~~about~~ 310 base ~~pairs~~ pairs.

35. (Previously presented) An isolated nucleic acid molecule comprising a sequence as set forth in SEQ ID NO: 11, or a degenerate variant thereof wherein said degenerate variant encodes the same amino acid sequence encoded by SEQ ID NO:11.

36. (Currently amended) A vector comprising the isolated nucleic acid molecule of either ~~any of~~ ~~claims 33-37~~ claims 33 or 34.

37. (Previously presented) A vector designated as pBVDdN6 (ATCC No. PTA-2532) (SEQ ID NO: 12).

38. (Currently amended) A cell transformed or transfected with any of the nucleic acid molecules of ~~any of claims 33-37~~ claims 33 or 34.

39. (Currently amended) A cell transformed or transfected with the vector of ~~claims 36 or 37~~ claim 36.

40. (Previously presented) A progeny virus produced by the cell of claim 38.

41. (Previously presented) A progeny virus produced by the cell of claim 39.

42. (Currently amended) A method of modifying the genomic nucleic acid molecule of an isolated wild type bovine viral diarrhea virus comprising:

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- (a) creating a mutated N<sup>pro</sup> coding sequence comprising an intact 5' region of at least ~~about~~ 36 base pairs, wherein said mutated N<sup>pro</sup> coding sequence encodes an inactive N<sup>pro</sup> protein; and
- (b) inserting a sequence coding for a monomeric bovine ubiquitin between the mutated N<sup>pro</sup> coding sequence and the coding sequence of the core protein.

43. (Currently amended) The method of claim 42 wherein the mutated N<sup>pro</sup> coding sequence comprises an intact 5' region of at least ~~about~~ 310 base ~~pairs~~ pairs.

44. (Currently amended) A method of attenuating an isolated wild type bovine viral diarrhea virus, comprising

- (a) isolating the genomic nucleic acid molecule from said virus,
- (b) creating a mutated N<sup>pro</sup> coding sequence comprising an intact 5' region of at least ~~about~~ 36 base pairs, wherein said mutated N<sup>pro</sup> coding sequence ~~encodes a an~~ encodes an inactive N<sup>pro</sup> protein; and;
- (c) inserting a sequence coding for a monomeric bovine ubiquitin between the mutated N<sup>pro</sup> coding sequence and the coding sequence of the core protein; and producing from the modified genome an attenuated virus suitable for use in a vaccine.

45. (Currently amended) The method of claim 44 wherein the mutated N<sup>pro</sup> coding sequence comprises an intact 5' region of at least ~~about~~ 310 base ~~pairs~~ pairs.

46. (Currently amended) An immunogenic composition comprising the attenuated bovine viral diarrhea virus of ~~any of claims 30-32~~ claims 30 or 32 and a veterinarily-acceptable carrier.

47. (Currently amended) An immunogenic composition comprising the isolated nucleic acid molecule of ~~any of claims 33-37~~ claims 33 or 34 and a veterinarily-acceptable carrier.

48. (Currently amended) A method of inducing an immune response against bovine viral diarrhea virus in an animal subject, comprising administering an immunologically effective amount of the

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attenuated bovine viral diarrhea virus of claims 30 or 32 ~~any of claims 30-32~~ and a veterinarily-acceptable carrier.

49. (Currently amended) A method of inducing an immune response against bovine viral diarrhea virus in an animal subject, comprising administering an immunologically effective amount of the isolated nucleic acid molecule of ~~any of claims 33-37~~ claims 33 or 34.

50. (Previously presented) The method of claim 48, wherein said immune response is a cellular or humoral immune response.

51. (Previously presented) The method of claim 49, wherein said immune response is a cellular or humoral immune response.

52. (Previously presented) The method of claim 48 wherein said immune response results in the production of antibodies against bovine viral diarrhea virus in said animal.

53. (Previously presented) The method of claim 49, wherein said immune response results in the production of antibodies against bovine viral diarrhea virus in said animal.

54. (Currently amended) A vaccine composition comprising the attenuated bovine viral diarrhea virus of claims 30 or 32 ~~any of claims 30-32~~ and a veterinarily-acceptable carrier.

55. (Currently amended) A vaccine composition comprising the isolated nucleic acid molecule of ~~any of claims 33-37~~ claims 33-34 and a veterinarily-acceptable carrier.

56. (Currently amended) A method of treating a bovine viral diarrhea virus infection in an animal, comprising administering to said animal, a therapeutically effective amount of the attenuated bovine viral diarrhea virus ~~any of claims 30-32~~ virus of claims 30 or 32.

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57. (Currently amended) A method of treating a bovine viral diarrhea virus infection in an animal, comprising administering to said animal, a therapeutically effective amount of the isolated nucleic acid molecule of ~~any of claims 33-37~~ claims 33 or 34.

58. (Previously presented) A method of identifying a bovine viral diarrhea virus in an animal as an attenuated bovine viral diarrhea virus of any of claims 30-32, said animal suspected of suffering a bovine viral diarrhea virus infection, comprising isolating the virus from said animal, detecting the presence of a ubiquitin coding sequence between a mutated N<sup>pro</sup> coding sequence and the coding sequence of the core protein, thereby determining the isolated virus as identical to the attenuated bovine viral diarrhea virus of claims 30-32.

59. (Previously presented) A method of identifying a bovine viral diarrhea virus in an animal as an attenuated bovine viral diarrhea virus of claims 30-32, said animal suspected of suffering a bovine viral diarrhea virus infection, comprising isolating the virus from said animal, detecting the presence of the mutation in the N<sup>pro</sup> gene, thereby determining the isolated virus as identical to the attenuated BVD virus of any of claims 30-32.

60. (New) The attenuated bovine viral diarrhea virus of claim 31 wherein the mutated N<sup>pro</sup> coding sequence comprises an intact 5' region of at least 310 base pairs.

61. (New) An isolated nucleic acid molecule comprising the genomic sequence of the attenuated bovine diarrhea virus of claim 60.

62. (New) A vector comprising the isolated nucleic acid molecule of claims 61.

63. (New) A cell transformed or transfected with the nucleic acid molecules of claim 61.

64. (New) A cell transformed or transfected with the vector of either claims 37 or 62.

65. (New) A progeny virus produced by the cell of claim 63.

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66. (New) A progeny virus produced by the cell of claim 64.
67. (New) An immunogenic composition comprising the attenuated bovine viral diarrhea virus of claims 31 or 60 and a veterinarily-acceptable carrier.
68. (New) An immunogenic composition comprising the isolated nucleic acid molecule of claims 35 or 61 and a veterinarily-acceptable carrier.
69. (New) A method of inducing an immune response against bovine viral diarrhea virus in an animal subject, comprising administering an immunologically effective amount of the attenuated bovine viral diarrhea virus of claims 31 or 60 and a veterinarily-acceptable carrier.
70. (New) A method of inducing an immune response against bovine viral diarrhea virus in an animal subject, comprising administering an immunologically effective amount of the isolated nucleic acid molecule of claims 35 or 61.
71. (New) The method of claim 69, wherein said immune response is a cellular or humoral immune response.
72. (New) The method of claim 70, wherein said immune response is a cellular or humoral immune response.
73. (New) A vaccine composition comprising the attenuated bovine viral diarrhea virus of claims 31 or 60 and a veterinarily-acceptable carrier.
74. (New) A vaccine composition comprising the isolated nucleic acid molecule of claims 35 or 61 and a veterinarily-acceptable carrier.

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75. (New) A method of treating a bovine viral diarrhea virus infection in an animal, comprising administering to said animal, a therapeutically effective amount of the attenuated bovine viral diarrhea virus of claims 31 or 60.

76. (New) A method of treating a bovine viral diarrhea virus infection in an animal, comprising administering to said animal, a therapeutically effective amount of the isolated nucleic acid molecule of claims 35 or 61.

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